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6. AUTHORS
Pamela A. Silver

5d. PROJECT NUMBER

5e. TASK NUMBER

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14. ABSTRACT
The overall goal of the grant is to create a synthetic biology platform based on solar energy that can be used on a local level to generate bulk amounts of chemical commodities. The specific aims of the original proposal are to construct a photosynthetic cynaobacterium that: 1) produces hydrogen by directed electron flow from the photosynthetic machinery; and 2) produces short alkyl chains by channeling electrons from photosynthesis into artificial metabolic pathways. During the course of the granting period, we also made significant progress on

15. SUBJECT TERMS
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a. REPORT	b. ABSTRACT	c. THIS PAGE			Pamela Silver
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Report Title

Final Report: Synthetic Biological Engineering of Photosynthesis

ABSTRACT

The overall goal of the grant is to create a synthetic biology platform based on solar energy that can be used on a local level to generate bulk amounts of chemical commodities. The specific aims of the original proposal are to construct a photosynthetic cynaobacterium that: 1) produces hydrogen by directed electron flow from the photosynthetic machinery; and 2) produces short alkyl chains by channeling electrons from photosynthesis into artificial metabolic pathways. During the course of the granting period, we also made significant progress on understanding the compartmentalization of carbon fixation and flux in relationship to photosynthesis and obtained increases in photosynthetic activity that have broad implications for production of commodities on demand.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
01/17/2012	8.00	Daniel_Ducat, Patrick_Boyle, Edwin_Wintermute, Jeffrey_Way, Christina_Agapakis, Pamela_Silver. Insulation of a synthetic hydrogen metabolism circuit in bacteria, Journal of Biological Engineering, (02 2010): 0. doi:
01/17/2012	9.00	Walter_Bonacci, Poh_Teng, Bruno_Afonso, Henrike_Niederholtmeyer, Patricia_Grob, Silver_Pamela, David_Savage. Modularity of a carbon-fixing protein organelle, PNAS, (01 2012): 0. doi:
08/16/2011	1.00	H. Niederholtmeyer, B. T. Wolfstadter, D. F. Savage, P. A. Silver, J. C. Way. Engineering Cyanobacteria To Synthesize and Export Hydrophilic Products, Applied and Environmental Microbiology, (04 2010): 0. doi: 10.1128/AEM.00202-10
08/16/2011	6.00	DC. Ducat , JC. Way , PA. Silver . Engineering cyanobacteria to generate high-value products, Trends in Biotechnology, (02 2011): 95. doi:
08/16/2011	5.00	CJ. Delebecque , AB. Lindner , PA. Silver , FA. Aldaye . Organization of intracellular reactions with rationally designed RNA assemblies., Science, (06 2011): 470. doi:
08/16/2011	3.00	Buz Barstow, Christina M Agapakis, Patrick M Boyle, Gerald Grandl, Pamela A Silver, Edwin H Wintermute. A synthetic system links FeFe-hydrogenases to essential E. coli sulfur metabolism, Journal of Biological Engineering, (05 2011): 5. doi: 10.1186/1754-1611-5-7
08/21/2012	12.00	G. Sachdeva, P. A. Silver, D. C. Ducat. Rewiring hydrogenase-dependent redox circuits in cyanobacteria, Proceedings of the National Academy of Sciences, (02 2011): 0. doi: 10.1073/pnas.1016026108
08/21/2012	2.00	Christina M. Agapakis, Pamela A. Silver. Modular electron transfer circuits for synthetic biology: insulation of an engineered biohydrogen pathway, Bioengineered Bugs, (12 2010): 413. doi: 10.4161/bbug.1.6.12462
08/21/2012	7.00	David_Savage, Bruno_Afonso, Anna_Chen, Pamela_Silver. Spatially Ordered Dynamics of the Bacterial Carbon Fixation Machinery, Science, (03 2010): 1258. doi:
08/21/2012	10.00	Daniel C. Ducat, J. Abraham Avelar-Rivas, Jeffrey C. Way, Pamela A. Silvera. Rerouting Carbon Flux To Enhance Photosynthetic Productivity, Applied and Environmental Microbiology, (03 2012): 2660. doi:
08/21/2012	11.00	Pamela A Silver, Daniel C Ducat. Improving carbon fixation pathways, Current Opinion in Chemical Biology, (08 2012): 0. doi: 10.1016/j.cbpa.2012.05.002
10/19/2015	15.00	Anna H. Chen, Avi Robinson-Mosher , David F. Savage , Pamela A. Silver , Jessica K. Polka. The bacterial carbon-fixing organelle is formed by shell envelopment of preassembled cargo, PLoS ONE, (09 2013): 76127. doi:

TOTAL: 12

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received	Paper
08/26/2013 14.00	Anna H. Chen, Avi Robinson-Mosher, David F. Savage, Pamela A. Silver, Jessica K. Polka. The Bacterial Carbon-Fixing Organelle is Formed by Shell Envelopment of Preassembled Cargo, Molecular Biology of the Cell (08 2013)
TOTAL:	**1**

Number of Manuscripts:

Books

Received	Book
TOTAL:	

Received	Book Chapter
TOTAL:	

Patents Submitted

Patents Awarded

Awards

2010-2013 Algae Biofuels Technical Advisory Committee, ExxonMobil Research
2010 IEE Award for most accessed paper in JBE
2010 Director, ARPA-E (DOE) grant in alternative electrofuels
2011 Nominee, ENI Award
2011-12 Fellow of the Radcliffe Institute for Advanced Study
2011- Elliot T. and Onie H. Adams Professorship of Biochemistry and Systems Biology, Harvard University
2012 The Tay Hayashi Lectureship, MBL
2013 Groundbreaking Science Speeches
2013 Top 20 Global Synthetic Biology Influencers

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Pamela A. Silver	0.01	
FTE Equivalent:	**0.01**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:...... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):...... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:...... 0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED
Kathy Buhl	0.01
FTE Equivalent:	**0.01**
Total Number:	**1**

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

Statement of Problem Studied

The overall goal of the grant is to create a synthetic biology platform based on solar energy that can be used on a local level to generate bulk amounts of chemical commodities.

Summary of most important results

Hydrogenases catalyze the reversible reaction 2H(+) + 2e(-) ¿ H(2) with an equilibrium constant that is dependent on the reducing potential of electrons carried by their redox partner. To examine the possibility of increasing the photobiological production of hydrogen within cyanobacterial cultures, we expressed the [FeFe] hydrogenase, HydA, from Clostridium acetobutylicum in the non-nitrogen-fixing cyanobacterium Synechococcus elongatus sp. 7942. We demonstrate that the heterologously expressed hydrogenase is functional in vitro and in vivo, and that the in vivo hydrogenase activity is connected to the light-dependent reactions of the electron transport chain. Under anoxic conditions, HydA activity is capable of supporting light-dependent hydrogen evolution at a rate > 500-fold greater than that supported by the endogenous [NiFe] hydrogenase. Furthermore, HydA can support limited growth solely using H(2) and light as the source of reducing equivalents under conditions where Photosystem II is inactivated. Finally, we demonstrate that the addition of exogenous ferredoxins can modulate redox flux in the hydrogenase-expressing strain, allowing for greater hydrogen yields and for dark fermentation of internal energy stores into hydrogen gas. These results proved our ability to connect electron flow from photosynthesis to hydrogen production. This accomplished Aim 1 and has broad significance for diverting electron flux in general.

One of our overarching goals was to engineer photosynthetic bacteria to produce commodities. We had a number of successes with regard to fatty acids. However, our biggest accomplishment was to engineer cyanobacteria to secrete sugar. This allows them to be used directly in bioreactors to feed cells producing more complex commodities. In addition, we made significant findings regarding how to increase the photosynthetic capacity that will have broad implications.

We have further characterized a strain of cyanobacteria that efficiently secretes sucrose at rates exceeding sugar production from terrestrial plants, providing a potential alternative feedstock source which does not compete with food crops for arable lands. The strain utilizes a sucrose symporter (cscB) in a fashion where the proton gradient across the cytoplasmic membrane is reversed, allowing secretion of sucrose produced autotrophically from solar energy and CO2. A substantial fraction of the carbon dioxide fixed by cscB-expressing S. elongatus can be exported as sucrose (50-85%), representing a highly efficient re-diversion of cellular resources. Sucrose is produced continuously and occurs at levels exceeding those previously reported for targeted production of metabolites in cyanobacteria and algae.

Furthermore, we described an unexpected improvement in photosynthetic productivity in sucrose-secreting cyanobacteria; results that have broad scientific implications for photosynthesis-driven production of a variety of valuable metabolites. We hypothesize that the export of this sugar feedstock acts to expand the cellular ‘metabolic sink’, allowing a greater utilization of solar energy under conditions of excess light. The engineered microalgae exhibit a 25-30% enhancement in photosynthetic activity relative to wild-type strains.

Finally, we demonstrated that the sucrose produced by this cyanobacterial strain can be utilized by industrially-relevant heterotrophic microbes (e.g. E. coli and S. cerevisiae) without further refinement or purification. We extended this research through the co-culture of cyanobacteria and a variety of heterotrophic microbes in order to evaluate the feasibility of indirect production of biofuels and other economically valuable bioindustrial compounds from solar energy.

Technology Transfer

Honors and Awards

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Scientific Progress and Accomplishments

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